Patent No. 5,518,918, which is a divisional of U.S. Patent Application Serial No. 07/962,522, filed October 16, 1992, now U.S. Patent No. 5,340,742, which is a continuation-in-part of U.S. Patent Application Serial No. 07/911,760, filed July 10, 1992, now U.S. Patent No. 5,340,594, which is a divisional of U.S. Patent Application Serial No. 07/580,778, filed September 11, 1990, now U.S. Patent No. 5,130,242, which is a continuation-in-part of U.S. Patent Application Serial No. 07/439,093, filed November 17, 1989, now abandoned, which is a continuation-in-part of U.S. Patent Application Serial No. 07/241,410, filed September 7, 1988, now abandoned, disclosures of which are incorporated by reference herein in their entirety.--

On page 9, line 4, please insert the following new paragraphs:

--Collection, isolation and selection of large numbers of suitable heterotrophic strains can be accomplished by the following method. Suitable water samples and organisms typically can be collected from shallow, saline habitats which preferably undergo a wide range of temperature and salinity variation. These habitats include marine tide pools, estuaries and inland saline ponds, springs, playas and lakes. Specific examples of these collection sites are: 1) saline warm springs such as those located along the Colorado river in Glenwood Springs, Colo., or along the western edge of the Stansbury Mountains, Utah; 2) playas such as Goshen playa located near Goshen, Utah; 3) marine tide pools such as those located in the Bird Rocks area of La Jolla, Calif.; and 4) estuaries, such as Tiajuana estuary, San Diego County, Calif. Special effort should be made to include some of the living plant matter and naturally occurring detritus (decaying plant and animal matter) along with the water sample. The sample can then be refrigerated until return to the laboratory. Sampling error is minimized if the water sample is shaken for 15-30 seconds, prior to pipetting or pouring a portion, for example, 1-10 ml, into a filter unit. The filter unit includes 2 types of filters: 1) on top, a sterile Whatman #4 filter (Trademark, Whatman Inc., Clifton, N.J.); and 2) underneath the Whatman filter, a polycarbonate filter with 1.0 μm pore size. The purpose of the first (top) filter is to remove all particulate matter greater than about 25 μm, generally allowing only unicellular type material to pass onto the 1.0 μm polycarbonate filter. The first filter greatly reduces the number of mold colonies that subsequently develop upon incubation of the polycarbonate filter at elevated temperatures, thereby enhancing the opportunities for other colonies to develop. Mold spores are

very numerous in coastal and inland saline waters, and mold colonies can quickly cover an agar plate unless screened out. The 1.0 μm size of the polycarbonate filter is chosen to allow many of the bacteria to pass on through into the filtrate. The purpose of using a sandwich filter design is to select for unicellular organisms at least a portion of whose cells range in diameter from about 1 μm to about 25 μm in size (organisms which could potentially be grown easily in a fermentor system for production on a large scale). Extensive growth of these unicellular organisms can be encouraged by incubation of the polycarbonate filter on an agar plate. Competition between organisms growing on the filter facilitates the isolation of competitive, robust strains of single-celled microorganisms. Unicellular aquatic microorganisms selected by the foregoing method display a range of cell size depending on growth conditions and stage of reproductive cycle. Most cells in culture have diameters in the range from about 1 μm to about 25 μm; however, cells (thalli and sporangia) in the cultures can be found that have larger diameters (depending on the strain) up to about 60 μm.

After filtration, the polycarbonate filter can be placed on an agar plate containing saline media containing a source of organic carbon such as carbohydrate including glucose, various starches, molasses, ground corn and the like, a source of assimilable organic or inorganic nitrogen such as nitrate, urea, ammonium salts, amino acids, microbial growth factors included in one or more of yeast extract, vitamins, and corn steep liquor, a source of assimilable organic or inorganic phosphorous, and a pH buffer such as bicarbonate. Microbial growth factors are currently unspecified compounds which enhance heterotrophic growth of unicellular microorganisms, including fungi and algae. The agar plates can be incubated in the dark at 25°-35°C. (30°C is preferred) and after 2-4 days numerous colonies will have appeared on the filter. Recovery of 1-5 colonies/plate of the desired organism is not uncommon. Yeast colonies are distinguishable either by color (they frequently are pink) or by their morphology. Yeast colonies are smooth whereas the desired organisms form in colonies with rough or textured surfaces. Individual cells of the desired organism can be seen through a dissecting microscope at the colony borders, whereas yeast cells are not distinguishable, due to their smaller size. Mold and higher fungi colonies are distinguishable from the desired organisms because they are filamentous, whereas the desired organisms are non-filamentous. Clear or white-colored colonies can be picked from the plates and restreaked on a new plate of similar media composition. While most of the desired organisms are clear or white-colored,

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some are orange or red-colored due to the presence of xanthophyll pigments and are also suitable for selection and restreaking. The new plate can be incubated under similar conditions, preferably at 30°C. and single colonies picked after a 2-4 day incubation period. Single colonies can then be picked and placed in, for example, 50 ml of liquid medium containing the same organic enrichments (minus agar) as in the agar plates. These cultures can be incubated for 2-4 days at 30°C with aeration, for example, on a rotary shaker table (100-200 rpm.). When the cultures appear to reach maximal density, 20-40 ml of the culture can then be harvested by centrifugation or other suitable method and preserved, as by lyophilization. The sample can then be analyzed by standard, well-known techniques including gas chromatography techniques to identify the fatty acid content of the strain. Those strains with omega-3 highly unsaturated fatty acids can thereby be identified and cultures of these strains maintained for further screening.

Promising strains can be screened for temperature tolerance by inoculating the strains into 250 ml shaker flasks containing 50 ml of culture media. These cultures are then incubated for 2 days on the shaker table over any desired temperature range from most practically between 27°-48°C, one culture at each 3°C interval. Production can be quantified as the total amount of fatty acids produced per ml of culture medium. Total fatty acids can be quantified by gas chromatography as described above. A similar process can also be employed to screen for salinity tolerance. For salinity tolerance a range of salinities yielding conductivities from 5-40 mmho/cm is adequate for most purposes. Screening for the ability to utilize a variety of carbon and nitrogen sources can also be conducted employing the procedure outlined above. The carbon and nitrogen sources were evaluated herein at concentrations of 5 g/l. Carbon sources evaluated were: glucose, corn starch, ground corn, potato starch, wheat starch, and molasses. Nitrogen sources evaluated were: nitrate, urea, ammonium, amino acids, protein hydrolysate, corn steep liquor, tryptone, peptone, or casein. Other carbon and nitrogen sources can be used, the choice being open to those of ordinary skill in the art, based on criteria of significance to the user.

IN THE CLAIMS:

Please cancel Claim 42 without prejudice to or disclaimer of the subject matter therein.